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A novel method for factor binding site mutagenesis

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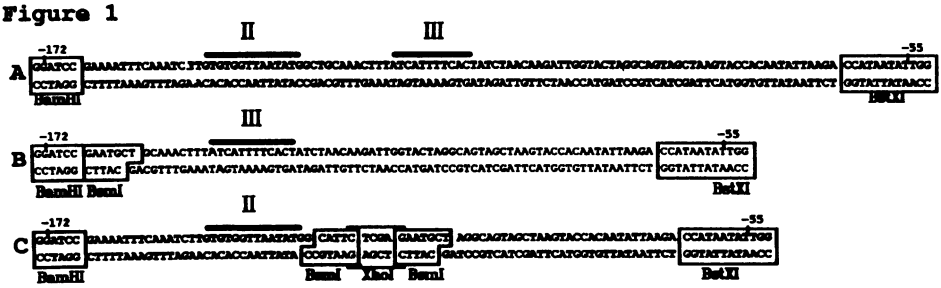
Current mutagenesis methods do not permit numerous defined base changes to be made simultaneously. Here we present a strategy with which specific, multiple mutations can be repeatedly introduced into a predefined region. This method is based on the ability of Type-IIS restriction enzymes to cut outside their recognition sites. This phenomenon has been exploited previously for other purposes (1,2). Our strategy involves the replacement of a region of interest, a factor binding site, with a cassette composed of divergent Type-IIS restriction enzyme sites. Figure 1 shows the creation of two such cassette replacements within the upstream region of the pea rbcS-3A gene (Fig.1A) (3). Sequences between restriction sites at -172 and -55 were replaced by oligonucleotides to recreate the upstream element with either a replacement of the binding site box II (Fig.1B) or box III (Fig.1C) (4). Cleavage of the cassettes with either BamHI/BsmI (Fig.1B), or BsmI (Fig.1C), creates non-complementary sticky ends derived from native sequence. Oligonucleotides containing specific base changes can be ligated unidirectionally into the vector to recreate a purely wild-type sequence with the exception of the desired changes. Construction of such vectors in plasmids containing an f1 origin enables direct sequencing of the mutant promoter elements. We believe that this strategy enhances the repertoire of techniques currently available for the analysis of DNA-protein interactions.

Figure 1



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References: 1) Szybalski, W. (1985) Gene 40, 169-173. 2) Mormeneo, S. et al (1987) Gene 61, 21-30. 3) Fluhr, R. et al (1987) EMBO J. 5, 2063-2071. 4) Green, P. et al (1987) EMBO J. 6, 2543-2549.